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## **Protective effects of sirtuins in cardiovascular diseases: from bench to bedside**

Winnik, Stephan ; Auwerx, Johan ; Sinclair, David A ; Matter, Christian M

**Abstract:** Sirtuins (Sirt1-Sirt7) comprise a family of nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent enzymes. While deacetylation reflects their main task, some of them have deacylase, adenosine diphosphate-ribosylase, demalonylase, glutarylase, and desuccinylase properties. Activated upon caloric restriction and exercise, they control critical cellular processes in the nucleus, cytoplasm, and mitochondria to maintain metabolic homeostasis, reduce cellular damage and dampen inflammation—all of which serve to protect against a variety of age-related diseases, including cardiovascular pathologies. This review focuses on the cardiovascular effects of Sirt1, Sirt3, Sirt6, and Sirt7. Most is known about Sirt1. This deacetylase protects from endothelial dysfunction, atherothrombosis, diet-induced obesity, type 2 diabetes, liver steatosis, and myocardial infarction. Sirt3 provides beneficial effects in the context of left ventricular hypertrophy, cardiomyopathy, oxidative stress, metabolic homeostasis, and dyslipidaemia. Sirt6 is implicated in ameliorating dyslipidaemia, cellular senescence, and left ventricular hypertrophy. Sirt7 plays a role in lipid metabolism and cardiomyopathies. Most of these data were derived from experimental findings in genetically modified mice, where NF B, Pcsk9, low-density lipoprotein-receptor, PPAR, superoxide dismutase 2, poly[adenosine diphosphate-ribose] polymerase 1, and endothelial nitric oxide synthase were identified among others as crucial molecular targets and/or partners of sirtuins. Of note, there is translational evidence for a role of sirtuins in patients with endothelial dysfunction, type 1 or type 2 diabetes and longevity. Given the availability of specific Sirt1 activators or pan-sirtuin activators that boost levels of the sirtuin cofactor NAD<sup>+</sup>, we anticipate that this field will move quickly from bench to bedside

DOI: <https://doi.org/10.1093/eurheartj/ehv290>

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ZORA URL: <https://doi.org/10.5167/uzh-155178>

Journal Article

Published Version

Originally published at:

Winnik, Stephan; Auwerx, Johan; Sinclair, David A; Matter, Christian M (2015). Protective effects of sirtuins in cardiovascular diseases: from bench to bedside. *European Heart Journal*, 36(48):3404-3412.

DOI: <https://doi.org/10.1093/eurheartj/ehv290>

## Clinical update

# Protective effects of sirtuins in cardiovascular diseases: from bench to bedside

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Received 21 April 2015; revised 19 May 2015; accepted 8 June 2015; online publish-ahead-of-print 25 June 2015

Sirtuins (Sirt1–Sirt7) comprise a family of nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent enzymes. While deacetylation reflects their main task, some of them have deacylase, adenosine diphosphate-ribosylase, demalonylase, glutarylase, and desuccinylase properties. Activated upon caloric restriction and exercise, they control critical cellular processes in the nucleus, cytoplasm, and mitochondria to maintain metabolic homeostasis, reduce cellular damage and dampen inflammation—all of which serve to protect against a variety of age-related diseases, including cardiovascular pathologies. This review focuses on the cardiovascular effects of Sirt1, Sirt3, Sirt6, and Sirt7. Most is known about Sirt1. This deacetylase protects from endothelial dysfunction, atherothrombosis, diet-induced obesity, type 2 diabetes, liver steatosis, and myocardial infarction. Sirt3 provides beneficial effects in the context of left ventricular hypertrophy, cardiomyopathy, oxidative stress, metabolic homeostasis, and dyslipidaemia. Sirt6 is implicated in ameliorating dyslipidaemia, cellular senescence, and left ventricular hypertrophy. Sirt7 plays a role in lipid metabolism and cardiomyopathies. Most of these data were derived from experimental findings in genetically modified mice, where NFκB, Pcsk9, low-density lipoprotein-receptor, PPARγ, superoxide dismutase 2, poly[adenosine diphosphate-ribose] polymerase 1, and endothelial nitric oxide synthase were identified among others as crucial molecular targets and/or partners of sirtuins. Of note, there is translational evidence for a role of sirtuins in patients with endothelial dysfunction, type 1 or type 2 diabetes and longevity. Given the availability of specific Sirt1 activators or pan-sirtuin activators that boost levels of the sirtuin cofactor NAD<sup>+</sup>, we anticipate that this field will move quickly from bench to bedside.

## Keywords

Sirtuins • Cardiovascular • Translational • Metabolism • Aging

## Sirtuins: mediators of caloric restriction

Silent Information Regulator 2 (SIR2) proteins—Sirtuins—are a family of histone deacetylases (HDACs) that catalyse deacetylation of both histone and non-histone lysine residues. Beyond deacetylase and deacylase activity, some sirtuins exert adenosine diphosphate (ADP)-ribosylase, demalonylase, desuccinylase, or glutarylase properties. Post-transcriptional modification of a wide range of protein targets of the seven mammalian orthologs (Sirt1–7) has been described in diverse settings. Their requirement for nicotinamide adenine dinucleotide (NAD<sup>+</sup>) distinguishes sirtuins from other HDAC classes and defines them as class III HDACs. This need of NAD<sup>+</sup> for their enzymatic activity closely links sirtuins to the

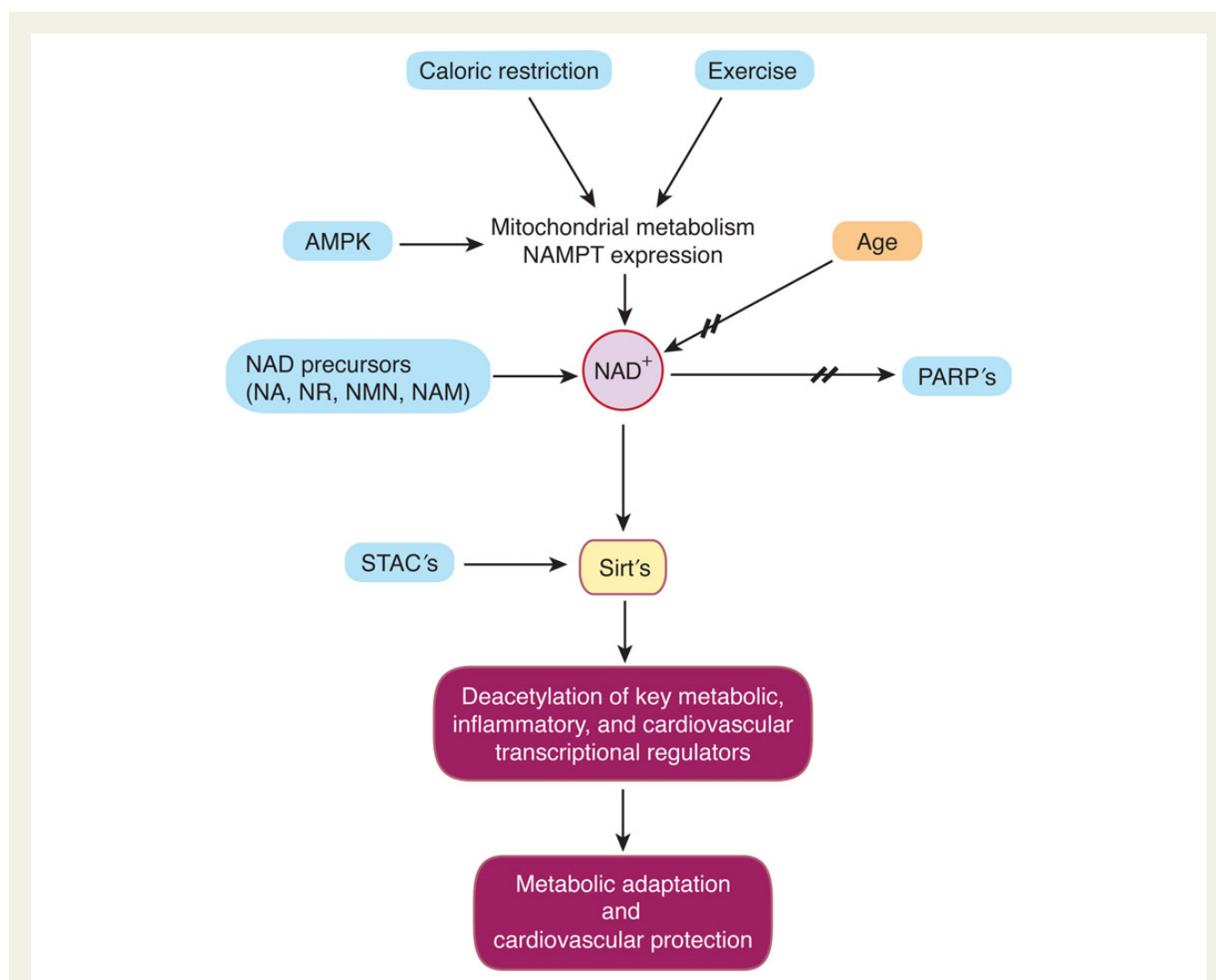
cellular energy status, increasing their activity at times of low energy availability such as caloric restriction and exercise (Figure 1).<sup>1–4</sup>

To date, caloric restriction is the most robust intervention that has been reproducibly shown to prolong life span and maintain health in both invertebrates and vertebrates, including mammals.<sup>5,6</sup> The first association between sirtuins and longevity in budding yeast 16 years ago<sup>7</sup> sparked efforts in numerous laboratories to unravel the mechanisms underlying sirtuin-mediated prolongation of life span and elucidate their potential to postpone the onset of age-related diseases. In this context, the seven mammalian sirtuin orthologs have been studied in diverse disease models, including insulin resistance and diabetes, inflammation, neurodegenerative disease, cancer, and more recently, in cardiovascular pathologies such as cardiac hypertrophy, heart failure, endothelial dysfunction, and atherosclerosis.

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**Figure 1** Specific and unspecific sirtuin activation. Caloric restriction, exercise, and increased activity of 5'-adenosine monophosphate activated protein kinase drive mitochondrial metabolism and expression of nicotinamide phosphoribosyltransferase. Consecutive synthesis of nicotinamide adenine diphosphate activates sirtuins. Another route of sirtuin activation is increased nicotinamide adenine diphosphate biosynthesis via supplementation of nicotinamide adenine diphosphate precursors such as nicotinic acid, nicotinamide, nicotinamide mononucleotide, and nicotinamide riboside or through inhibition of nicotinamide adenine dinucleotide-consuming activities, such as poly-adenosine diphosphate-ribose-polymerases or CD38. Sirt1-activating compounds may mimic the effects of Sirt1. Stimulation of Sirt1 activity by these distinct pathways improves the capacity of the cells/organism for metabolic adaptation and/or cardiovascular protection.

This review focuses on the recent advances of sirtuin research in experimental cardiovascular disease and discusses their potential clinical applications (Table 1).

## Sirtuins: emerging roles in cardiovascular and metabolic diseases

### Atherosclerosis: a chronic immunometabolic disease

Atherosclerosis with its sequelae myocardial infarction or stroke comprises the major cause for morbidity and mortality in the Western world.<sup>8</sup> At the cellular and molecular level, atherosclerosis

results from a complex interplay between modified low density lipoproteins (LDL), activated endothelial cells, monocyte-derived macrophages, T cells, and the vessel wall: Oxidative stress in endothelial cells, endothelial dysfunction, and subsequent recruitment of macrophages into the sub-endothelial space are major steps in early atherogenesis. In response to para- and autocrine inflammatory mediators such as tumour necrosis factor  $\alpha$  and different interleukins, invading monocytes transdifferentiate into macrophages, proliferate, and ingest abundantly present modified LDL-cholesterol (LDL-C). Thereby, sub-endothelial macrophages turn into foam cells that promote plaque formation and subsequent plaque rupture.<sup>9</sup> Plasma cholesterol and in particular its LDL subfraction are central players for both the initiation and progression of atherosclerosis.<sup>10</sup>

**Table 1** Cardiovascular and metabolic effects of sirtuins and pan-sirtuin activators; genetic and drug interventions: gain- and loss-of-function in animals and humans

Sirtuin	Model	Key findings: mediators	Reference
Experimental			
Sirt1		Sirt1 LOF: ↑ angiogenesis; endothelial cells	Potente et al. <sup>25</sup>
Sirt1	ApoE <sup>-/-</sup>	Sirt1 EC GOF: ↓ atherosclerosis	Zhang et al. <sup>12</sup>
Sirt1	ApoE <sup>-/-</sup>	Sirt1 LOF: ↑ atherosclerosis; foam cells; Lox1, NFκB	Stein et al. <sup>13</sup>
Sirt1	ApoE <sup>-/-</sup>	Sirt1 LOF: ↑ endothelial activation (VCAM-1), NFκB	Stein et al. <sup>14</sup>
Sirt1	WT	Sirt1 LOF: ↑ thrombosis; endothelial cells, NFκB	Breitenstein et al. <sup>20</sup>
Sirt1	LDLR <sup>-/-</sup>	Sirt1 GOF: ↑ atherosclerosis; SREB in liver	Qiang et al. <sup>23</sup>
Sirt1	ApoE <sup>-/-</sup>	Sirt1 GOF in VSMC: ↓ atherosclerosis	Gorenne et al. <sup>16</sup>
Sirt1	ApoE <sup>-/-</sup>	STAC (SRT3025): ↓ atherosclerosis, PCSK9, LDLR	Miranda et al. <sup>22</sup>
Sirt3		Sirt3 GOF: ↓ LVH	Sundaresan et al. <sup>39</sup>
Sirt3	ApoE <sup>-/-</sup>	Sirt3 LOF: = atherosclerosis, ↓ metabolic flexibility	Winnik et al. <sup>37</sup>
Sirt3	TAC	Sirt3 LOF: ↑ LVH with fibrosis	Hafner et al. <sup>40</sup>
Sirt6		Sirt6 LOF: accelerated aging—hypoglycaemia	Mostoslavsky et al. <sup>60</sup>
		Sirt6 LOF: ↑ LVH;	Sundaresan et al. <sup>50</sup>
		Sirt6 GOF: ↓ LVH	
		Sirt6 Hep LOF: ↑ PCSK9, plasma LDL-cholesterol	Tao et al. <sup>55</sup>
		Sirt6 Hep GOF: ↓ PCSK9, plasma LDL-cholesterol	
Sirt7		Sirt7 LOF: ↑ CMP, liver steatosis, TG	Ryu et al. <sup>66</sup>
		Sirt7 LOF: ↑ LVH, inflammatory CMP, fibrosis	Vakhrusheva et al. <sup>97</sup>
PanSirt			
NAD <sup>+</sup>	Aging	NAD <sup>+</sup> booster in old mice: restores mitochondrial function and metabolic abnormalities	Gomes et al. <sup>83</sup>
NR	DIO	NAD <sup>+</sup> booster in DIO: improves metabolic abnormalities	Canto et al. <sup>88</sup>
NR	Aging	NAD <sup>+</sup> booster in old mice: restores mitochondrial function	Mouchiroud et al. <sup>84</sup>
Exercise	Aging	Exercise in old rats: Increases NAD <sup>+</sup> , NAMPT, Sirt1, Sirt6	Koltai et al. <sup>86</sup>
Clinical			
Sirt1	Adults	Sirt1 SNPs: ↓ energy expenditure in offspring of T2D pts	Lagouge et al. <sup>19</sup>
Sirt1	ACS	Monocytes in ACS pts: ↓ Sirt1 expression than in healthy	Breitenstein et al. <sup>70</sup>
Sirt1	Adults	Carotid atherosclerosis: ↓ Sirt1 expression	Gorenne et al. <sup>16</sup>
Sirt1	Adults	Smokers: STAC (SRT2104): ↓ endothelial dysfct, LDL-C	Venkatasubramanian et al. <sup>82</sup>
Sirt6	Adults	Failing hearts: ↓ Sirt6 expression	Sundaresan et al. <sup>50</sup>

Experimental and human data showing cardiovascular effects of sirtuin modulation; LOF, loss-of-function; GOF, gain-of-function; DIO, diet-induced obesity; SRT3025, SRT2104; STAC, Sirt1-activating compound; CMP, cardiomyopathy; LVH, left ventricular hypertrophy; ACS, acute coronary syndromes; NAD<sup>+</sup>, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NR, nicotinamide riboside; T2D, type 2 diabetes; pts, patients.

It is intriguing that many risk factors for cardiovascular disease such as age, hypercholesterolaemia, obesity, and type 2 diabetes are conditions in which the activation of the sirtuins has been shown to exert protective effects in experimental models.

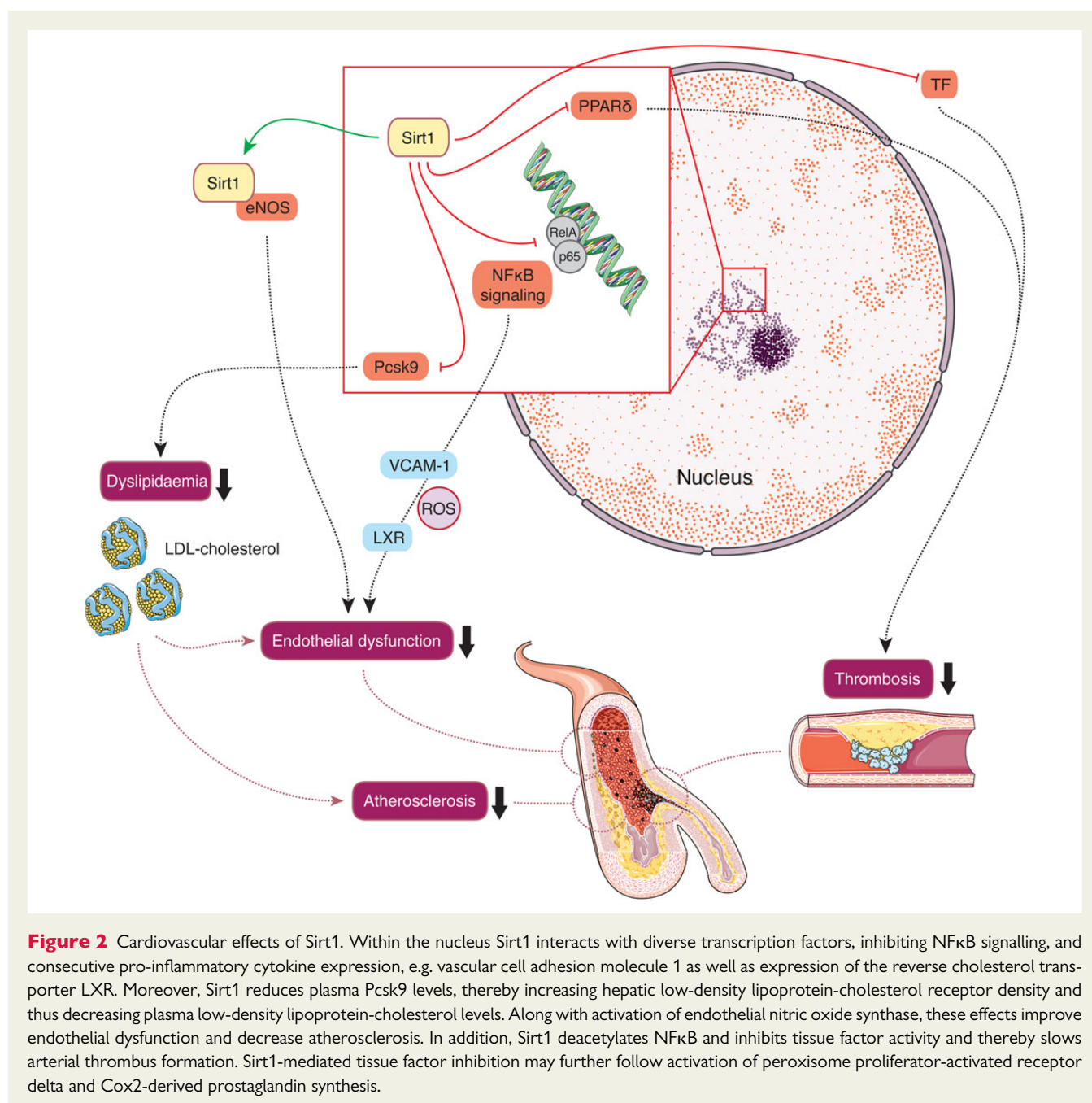
## Sirt1 in cardiovascular disease

Sirt1 is found in the nucleus and cytoplasm (Figure 2). Among all seven sirtuin isoforms, most is known about Sirt1. First evidence of a connection between Sirt1 and endothelial cells was that Sirt1 activates endothelial nitric oxide synthase (eNOS).<sup>11</sup> Later on, studies in genetically engineered mouse models have demonstrated that Sirt1 exerts atheroprotective effects by activating eNOS or by diminishing NFκB activity in endothelial cells and macrophages.<sup>12–14</sup> Moreover, pharmacological Sirt1 activation protected endothelial cells from senescence induced by disturbed flow.<sup>15</sup> Another report assigned Sirt1 in vascular smooth muscle cells a protective role against DNA damage, medial degeneration, and atherosclerosis.<sup>16</sup>

These reports place macrophages, endothelial, and vascular smooth muscle cells at centre stage for Sirt1-mediated atheroprotection.<sup>17</sup>

Sirt1 also has wide-ranging effects on metabolic homeostasis, mainly through its role as a master regulator of mitochondrial integrity.<sup>18,19</sup> Sirt1 activation hence results in improved glucose tolerance and lipid homeostasis and reduced inflammatory tone, which all are also atheroprotective.<sup>1,2</sup>

Pharmacological Sirt1 inhibition has been reported to increase thrombosis by inhibiting tissue factor activation via NFκB.<sup>20</sup> Similarly, cyclooxygenase-2-derived prostacyclin and PPARδ activation were found to decrease arterial thrombus formation by suppressing tissue factor in a sirtuin-1-dependent manner.<sup>21</sup> Thus, activation of Sirt1 protects from arterial thrombosis. In the context of atherosclerosis, pharmacological Sirt1 activation lowered plasma LDL-C levels by inhibiting proprotein convertase subtilisin/kexin 9 (Pcsk9) secretion, thereby increasing hepatic LDL-receptor (LDL-R) availability and consecutive LDL-C clearing.<sup>22</sup> Conversely, absence of LDL-R



abolished the atheroprotective effects of pharmacological Sirt1 activation.<sup>22</sup> These experiments explained the controversial finding that genetic Sirt1 overexpression increased atherosclerosis in LDL-R knockout mice.<sup>23</sup> Moreover, Sirt1 was reported to promote angiogenesis via inhibition of endothelial notch signaling.<sup>24,25</sup>

Sirt1 also provides cardiac protection: *Sirt1*-deficient mice exhibit increased injury in response to ischaemia-reperfusion, whereas injury was decreased in Sirt1 transgenic mice.<sup>26</sup> Moreover, Sirt1 was shown to protect from catecholamine-induced cardiomyopathy in mice.<sup>27</sup>

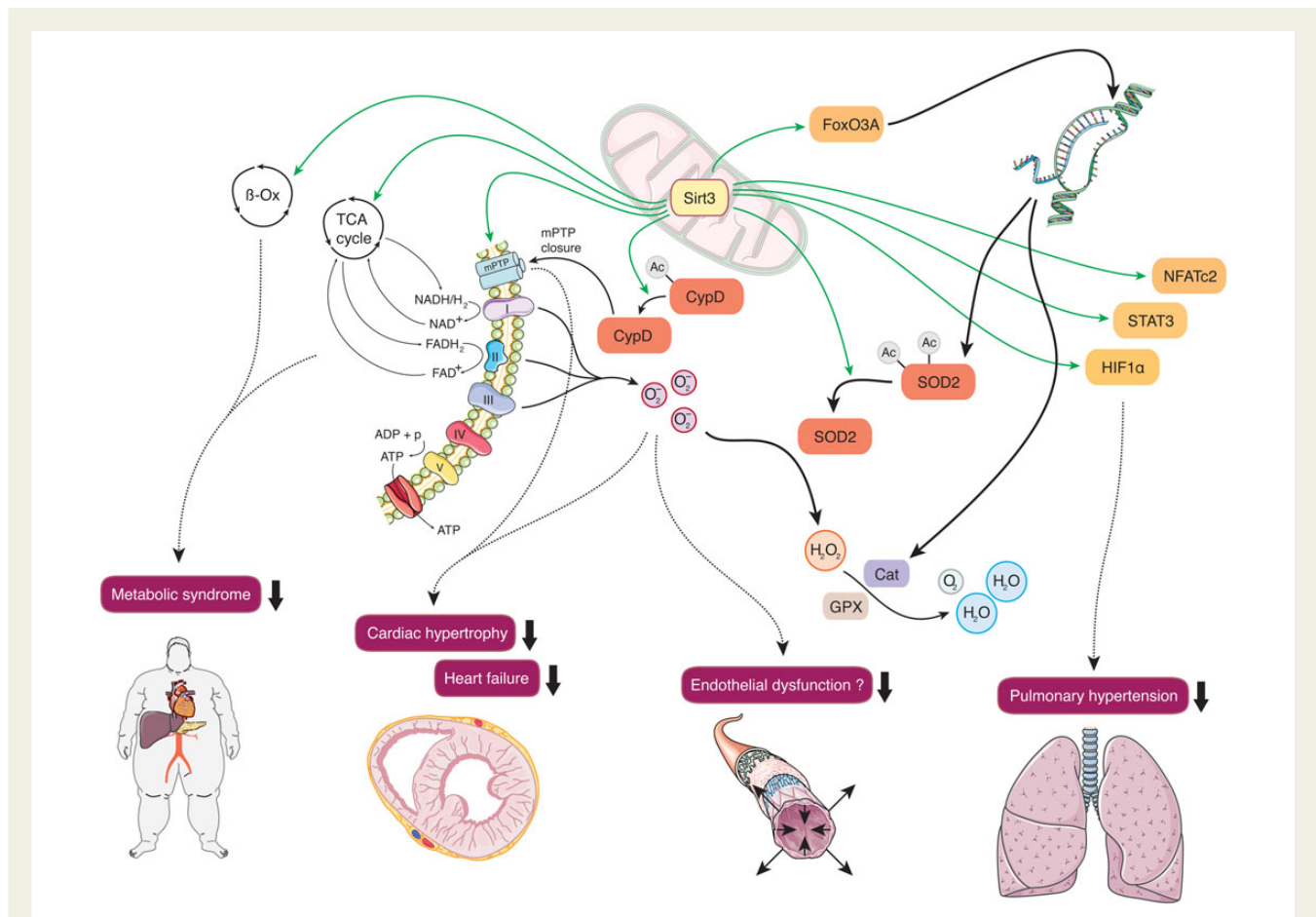
### Sirt3 in cardiovascular disease

Together with Sirt4 and Sirt5, Sirt3 is located in the mitochondria (Figure 3). Sirt3 regulates global mitochondrial lysine acetylation,<sup>28</sup>

boosting antioxidant defense, and preserving mitochondrial function.<sup>29–32</sup> Mitochondrial dysfunction plays a central role in a number of cardiovascular diseases, ranging from hypertrophic and dilated cardiomyopathy, heart failure, and pulmonary hypertension to endothelial dysfunction in early atherogenesis.<sup>33–35</sup>

The protective effects of caloric restriction on oxidative stress depend on the presence of Sirt3, which increased the ratio of glutathione to glutathione disulphide.<sup>36</sup> Similarly, deletion of *Sirt3* was associated with increased malondialdehyde levels in LDL-R knockout mice, yet without affecting atherogenesis.<sup>37</sup> Concordantly, overexpression of *Sirt3* decreased cellular levels of reactive oxygen species (ROS), an effect that was brought about by Sirt3-mediated deacetylation and activation of superoxide dismutase 2 (SOD2), the





**Figure 3** Cardiovascular effects of Sirt3. Mitochondrial Sirt3 drives the tricarboxylic acid cycle,  $\beta$ -oxidation, and oxidative phosphorylation, thus maintaining metabolic homeostasis and preventing the development of risk factors associated with the metabolic syndrome. Deacetylation and consecutive activation of superoxide dismutase 2 mediates antioxidative protection, diminishes cardiac hypertrophy, and may improve endothelial dysfunction. Activation of the transcription factors NFATc2, STAT3, and HIF1 $\alpha$  prevent the development of pulmonary hypertension.

mitochondrial isoform of this group of major ROS detoxifying enzymes.<sup>36,38</sup> Importantly, excessive ROS, subsequent mitochondrial DNA damage, and progressive respiratory chain dysfunction activate several signalling pathways underlying endothelial dysfunction and vascular inflammation in atherogenesis.<sup>33</sup> Sirt3-dependent transcriptional induction of SOD2 and catalase was shown to prevent cardiac hypertrophy by deacetylation of the transcription factor forkhead box O3a (Foxo3a).<sup>39</sup> Moreover, *Sirt3*-deficient mice were more susceptible to age-dependent and trans aortic constriction-induced left ventricular hypertrophy via activation of the mitochondrial permeability transition pore.<sup>40</sup> Sirt3 was also reported to prevent stress-induced mitochondrial apoptosis of mammalian cardiomyocytes and to protect endothelial mitochondria from oxidative damage.<sup>31,41</sup>

Interestingly, *Sirt3* knockout mice develop spontaneous pulmonary hypertension.<sup>42</sup> Moreover, mitochondria isolated from pulmonary artery smooth muscle cells of *Sirt3*-deficient mice displayed a reduced oxygen consumption rate compared with controls. Concordantly, *Sirt3* deficiency in both murine and human pulmonary artery smooth muscle cells was associated with an induction of the three transcription factors HIF1 $\alpha$ , STAT3, and NFATc2, all

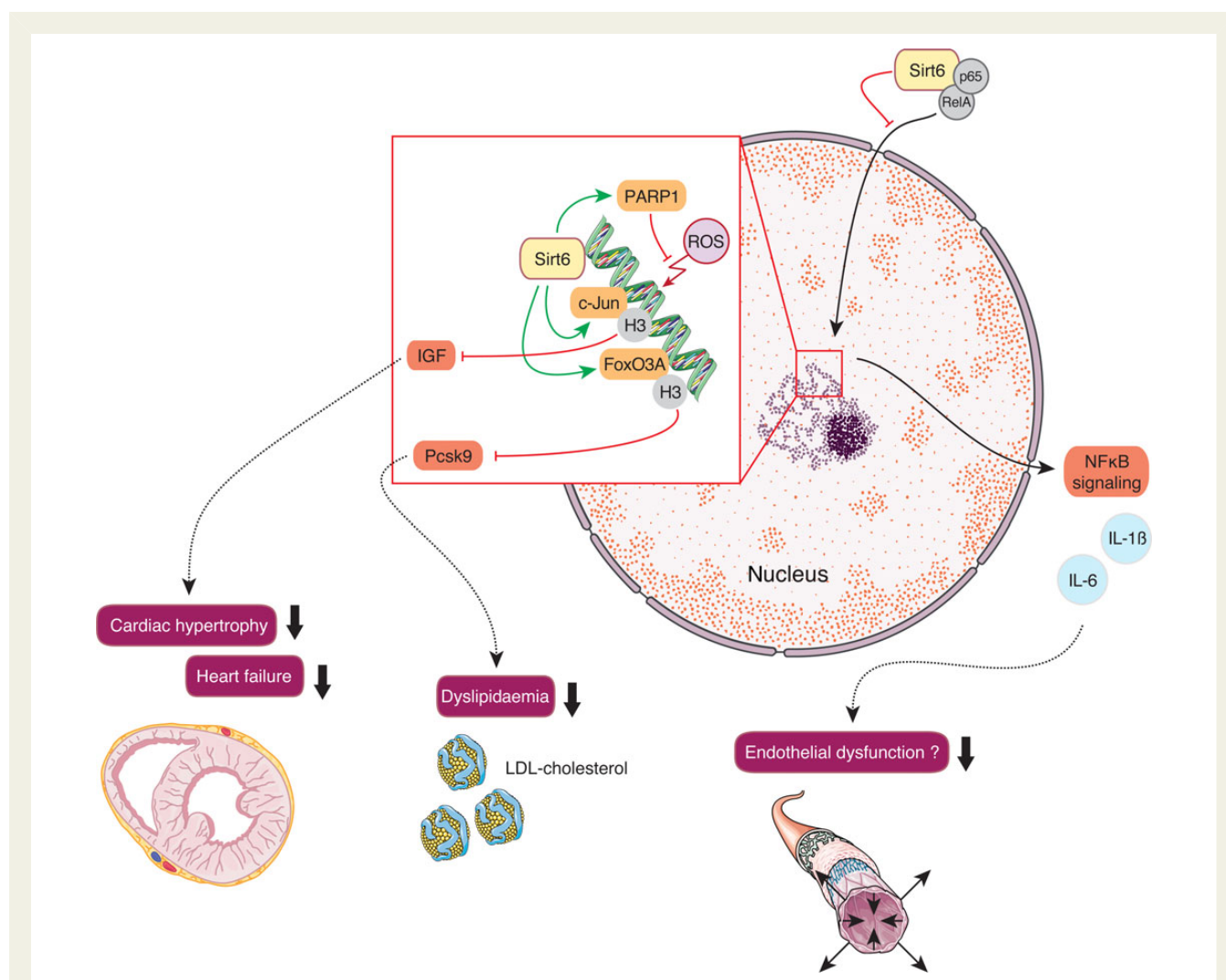
known to be essential in the development of pulmonary arterial hypertension.<sup>42</sup>

Sirt3 has been shown to orchestrate mitochondrial metabolism by driving not only oxidative phosphorylation<sup>29,43,44</sup> but also the tricarboxylic acid cycle,<sup>32</sup> and  $\beta$ -oxidation.<sup>45</sup> In this context, *Sirt3* deficiency accelerated the development of the metabolic syndrome in mice, a cluster of risk factors for cardiovascular diseases.<sup>37,46</sup>

### Sirt6 in cardiovascular disease

Sirt6 is a nuclear chromatin-associated deacetylase (Figure 4). Recent reports provided mechanistic insight on the ADP-ribosyltransferase substrate-specific deacetylase activity of both acetyl and long-chain fatty-acyl groups.<sup>47</sup> Sirt6 plays a role in cardiovascular disease including cardiac hypertrophy, heart failure, myocardial hypoxic damage, and metabolism.<sup>48–50</sup> Sirt6 has gained attention for its role in human telomere and genome stabilization,<sup>51</sup> gene expression and DNA repair,<sup>52</sup> glucose and fat homeostasis,<sup>53–55</sup> and inflammation.<sup>56,57</sup>

Sirt6 contributes to chromosomal stability by promoting double-strand break repair by homologous recombination (HR).<sup>58</sup> DNA double-strand breaks are a hallmark of genomic instability in aging tissues. Notably, overexpression of Sirt6 in pre-senescent cells



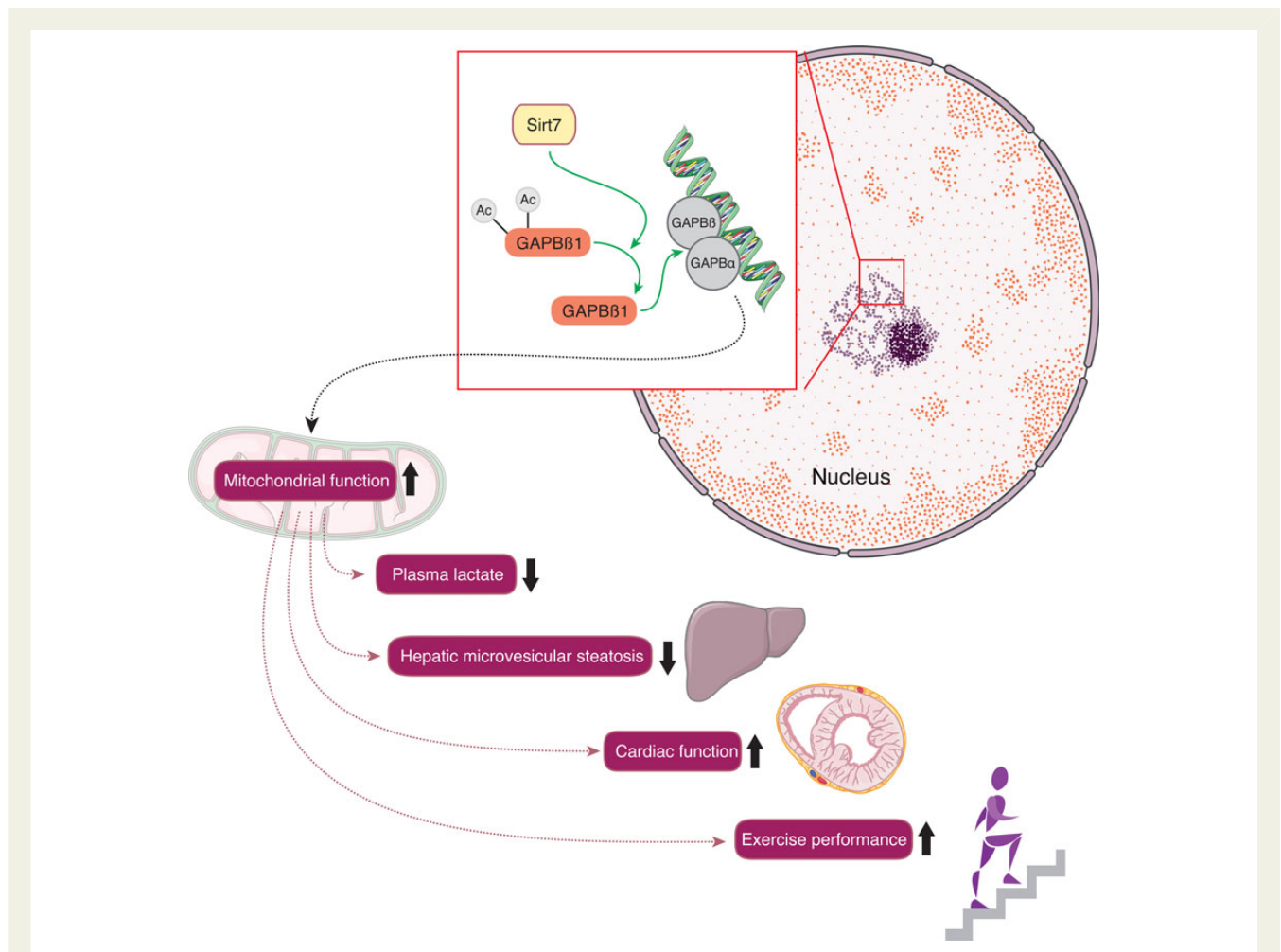
**Figure 4** Cardiovascular effects of Sirt6. Nuclear Sirt6-induced inhibition of insulin-like growth factor signalling prevents cardiac hypertrophy. Moreover, sirtuin 6-mediated reduction of plasma Pcsk9 levels increases hepatic low-density lipoprotein-receptor density and decreases plasma low-density lipoprotein-cholesterol levels. Poly[adenosine diphosphate-ribose] polymerase 1 activation halts reactive oxygen species-mediated DNA damage. Moreover, physical interaction of Sirt6 with the NFκB subunits RelA and p65 prevents their translocation to the nucleus and inhibits pro-inflammatory NFκB-signalling, thereby potentially protecting from endothelial dysfunction.

stimulated HR.<sup>58</sup> This support in HR can be partially attributed to a physical interaction and consecutive stimulation of Sirt6 with poly[ADP ribose] polymerase 1, thereby preventing the premature aging phenotype of *Sirt6* knockout mice.<sup>59,60</sup> Concomitantly, male *Sirt6* transgenic mice revealed an increased life span compared with *wild-type* controls.<sup>54</sup> Interestingly, the heart is among the organs with the highest Sirt6 expression, suggesting a role for Sirt6-mediated protection from myocardial senescence.<sup>61</sup>

Indeed, Sirt6 was identified as a negative endogenous regulator of myocardial IGF-Akt signaling, a pathway that upon constitutive activation eventually leads to cardiac hypertrophy. *Sirt6* deficiency enhanced H3K9 acetylation, facilitating binding of the stress-responsive transcription factor c-Jun. Subsequent boosting of myocardial IGF signalling resulted in cardiac hypertrophy and heart failure.<sup>50</sup> Along this line, nicotinamide mononucleotide adenylyltransferase, a vital enzyme in NAD biogenesis, prevented angiotensin II-induced cardiac

hypertrophy.<sup>48</sup> Moreover, cardiomyocytes from *Sirt6* transgenic mice were protected from prolonged hypoxia *ex vivo*, an effect that was in part attributed to increased Sirt6-mediated deacetylation and inactivation of RelA (p65), a subunit of NFκB.<sup>49</sup>

By the transcriptional repression of NFκB-dependent targets, Sirt6 plays an important role in stress response, thereby protecting against inflammatory and degenerative diseases.<sup>56,57</sup> Importantly, this interaction with NFκB-signalling may contribute to premature senescence and early lethality upon *Sirt6* deficiency.<sup>62,63</sup> Vascular inflammation is a key regulatory process in atherogenesis.<sup>64</sup> Thus, *Sirt6* deficiency in cultured endothelial cells increased expression of pro-inflammatory cytokines such as interleukin 1β along with an increased transcriptional activity of NFκB.<sup>65</sup> Intriguingly, hepatic Sirt6 was also shown to suppress transcription of Pcsk9, thereby preventing hepatic LDL-R degradation and consecutively reducing plasma LDL-C levels in mice.<sup>55</sup> Increased levels of LDL-C in concert



**Figure 5** Cardiovascular effects of Sirt7. Nuclear Sirt7 deacetylates distinct lysine residues located in the hetero- and homodimerization domains of GA-binding protein (GABP)β1, a master regulator of nuclear-encoded mitochondrial genes. Along these lines, Sirt7 improves mitochondrial function in numerous tissues including cardiac and skeletal muscle where it protects from cardiomyopathy, lowers lactate levels, and improves exercise performance, respectively. Moreover, Sirt7 protects from hepatic micro-vesicular steatosis.

with enhanced pro-inflammatory cytokines trigger endothelial activation and launch the vicious immunometabolic cycle of atherogenesis.

### Sirt7 in cardiovascular disease

Data from two different laboratories, using two independently generated mouse lines have also linked Sirt7 with cardiomyopathy, the main cause of death of Sirt7-deficient mice (Figure 5).<sup>66,67</sup> In fact, Sirt7 deficiency in mice induces multi-systemic mitochondrial dysfunction, which is reflected by increased blood lactate levels, reduced exercise performance due to cardiac dysfunction, hepatic micro-vesicular steatosis, and age-related hearing loss.<sup>66,68</sup> This link between Sirt7 and mitochondrial function can be translated to humans, where Sirt7 overexpression rescues the mitochondrial functional defect in fibroblasts of patients with a mutation in NADH dehydrogenase [ubiquinone] iron-sulphur protein 1. These wide-ranging effects of Sirt7 on mitochondrial homeostasis are the consequence of the deacetylation of distinct lysine residues located in the hetero- and homodimerization domains of GA-binding

protein (GABP)β1, a master regulator of nuclear-encoded mitochondrial genes. Sirt7-mediated deacetylation of GABPβ1 facilitates complex formation with GABPα and the transcriptional activation of the GABPα/GABPβ heterotetramer. These data suggest that Sirt7 is a dynamic nuclear regulator of mitochondrial function through its impact on GABPβ1. The role of Sirt7 in endothelial function and atherothrombosis remains unknown.

### Sirtuins: a role in human cardiovascular disease

Data on sirtuins in human cardiovascular diseases are scarce. Of note, reports about associations have to be differentiated from findings related to potential causal effects.

Most studies report lower sirtuin expression levels in contexts of cardiovascular disease compared with healthy controls. However, none of them tracked specific sirtuin activity. First, data were derived from patients with insulin resistance: Low Sirt1 expression in insulin-sensitive tissues correlated with impaired stimulation of



energy expenditure by insulin. Thus, these findings associated impaired regulation of mitochondrial function with insulin resistance in humans.<sup>69</sup> Additional reports came from patients with atherosclerosis: Sirt1 expression levels were found to be lower in human atherosclerotic carotid arteries compared with non-diseased arteries.<sup>16</sup> Sirt1 expression levels were also lower in monocytes of patients with acute coronary syndromes compared with healthy controls.<sup>70</sup> In parallel, Sirt6 expression and inflammatory activity in diabetic atherosclerotic plaques increased upon incretin treatment and was associated with a more stable plaque phenotype.<sup>71</sup>

In contrast to associative reports, genetic studies imply a more stringent cause–effect relationship. A report in Finnish subjects demonstrated a direct involvement of Sirt1 in modulating energy expenditure and metabolic homeostasis: Three of five single-nucleotide polymorphisms (SNPs) were significantly associated with whole body energy expenditure as evaluated either during fasting or during a hyperinsulinemic clamp.<sup>19</sup> Another study showed that a point mutation of Sirt1 was associated with human type 1 diabetes and ulcerative colitis; expression of this mutation in insulin-producing cells resulted in overproduction of nitric oxide and pro-inflammatory cytokines.<sup>72</sup> Yet, no association was observed between five known SNPs of the *Sirt1* gene with longevity using an extensive DNA data bank of 1573 long-lived individuals with matched younger controls.<sup>73</sup> Of note, many other studies reporting SNPs confer limitations such as low patient numbers or insufficient adjustments in control groups.

Moreover, Sirt3 deficiency was associated with pulmonary arterial hypertension and the metabolic syndrome both in mice and humans.<sup>42,46</sup> Sirt6 levels were decreased in human failing hearts<sup>50</sup>; in addition, genetic variants of Sirt6 and uncoupling protein 5 were associated with carotid atherosclerotic plaque burden.<sup>74</sup> To date, there are no reports about genetic variants of Sirt7 and human disease.

## Pharmacological modulation of sirtuin activity

Two reports that resveratrol protected mice against diet-induced obesity and insulin resistance with Sirt1 as a crucial mediator created considerable attention.<sup>18,19</sup> These effects of resveratrol supplementation, mimicking caloric restriction, could later on be translated into humans.<sup>75</sup> Subsequently, more specific Sirt1-activating compounds (STACs) applied in experimental diet-induced obesity mimicked the protective effects of caloric restriction, showing mitochondrial activation, and subsequent prevention of obesity along with an improvement of the diabetic phenotype.<sup>76,77</sup> Moreover, STAC improved survival and healthspan in obese mice.<sup>78</sup>

However, the specificity of these initial STAC was called into question: A non-physiological fluorescent substrate that was used for the Sirt1 activity assays was shown to lead to artefactual results.<sup>79</sup> Nevertheless, the substrate specificity of STAC was consecutively underlined: Mutation of a single amino acid in Sirt1, Glu<sup>230</sup> located in a structured N-terminal domain was critical for Sirt1 activation.<sup>80</sup> Given the conservation of Glu<sup>230</sup> down to model organisms (*drosophila*), the presence of an endogenous activator appears logical, which however remains to be proven. Furthermore,

the most recent pharmacological STAC have been used with beneficial effects in mice for atheroprotection and extension of lifespan,<sup>22,81</sup> as well as in healthy smokers for improving endothelial dysfunction and lowering LDL-C.<sup>82</sup>

While Sirt1-specific activators may ultimately prove operative,<sup>22,80</sup> there is increasing evidence that pan-sirtuin activators might be more effective.<sup>83–85</sup> The metabolite NAD<sup>+</sup> is an essential co-substrate for the activity of all sirtuins. Its levels decline in response to high-fat diet, DNA damage, and aging.<sup>84</sup> Reports on sirtuin-dependent beneficial effects of increasing NAD<sup>+</sup> levels on metabolic homeostasis suggest that this strategy provides a novel and promising concept for cardiovascular protection.

NAD<sup>+</sup> levels are maintained by balancing its biosynthesis/salvage and breakdown (Figure 1). NAD<sup>+</sup> content can be boosted by exercise,<sup>4,86</sup> inhibition of NAD<sup>+</sup>-consuming enzymes [sirtuins, poly-ADP-ribose-polymerases (PARPs),<sup>87</sup> and cyclic ADP-ribose synthases], and administration of NAD<sup>+</sup> precursors, such as nicotinic acid, nicotinamide, nicotinamide mononucleotide,<sup>83</sup> and nicotinamide riboside.<sup>84,88</sup> NAD<sup>+</sup> precursor compounds as well as PARP and CD38 inhibitors have been used in several long-term mouse studies confirming wide-ranging health benefits on the metabolic, immune, and nervous systems.<sup>89</sup> Raising NAD<sup>+</sup> levels may improve cardiac function in the context of ischaemia-reperfusion,<sup>90</sup> but may be damaging in the setting of acute myocardial infarction.<sup>91</sup> Thus, the net effects of increasing NAD<sup>+</sup> levels are likely to be dose- and time-dependent; such a biphasic response is well known from nitric oxide. The effects of raising NAD<sup>+</sup> levels long term in the context of chronic cardiovascular disease are likely to be beneficial. Yet, this has to be formally tested.

## Sirtuins in cardiovascular diseases: conclusions and perspectives

The beneficial effects of sirtuins on inflammation, lipid metabolism, and numerous areas of cardiovascular disease are well documented at the pre-clinical level. The prognostic value of deranged pathways of inflammation and lipids for human cardiovascular disease is established.<sup>92,93</sup> Multi-omics approaches will enlarge our instrumentarium of diagnostic and prognostic biomarkers in cardiovascular diseases.<sup>94</sup> Moreover, large databases for linking genotypes to cardiovascular phenotypes and prognosis are available.<sup>95</sup> Yet, the tracking of specific sirtuin activity remains a challenge.<sup>96</sup> Some genetic reports imply a cause–effect relationship of Sirt1 SNPs with metabolic homeostasis. Yet, conclusive genetic analyses from large databases (GWAS) about the role of Sirt1 SNPs are still pending. At the experimental level, both specific Sirt1 activators and pan-sirtuin-activating compounds are in development and hold great promise for future applications in treating and preventing cardiovascular and metabolic diseases.

## Funding

This work was funded by the Swiss National Science Foundation (J.A., C.M.M.), the Ecole Polytechnique Fédérale de Lausanne (J.A.), the NIH/NIA (D.A.S., J.A.), the Paul Glenn Foundation for Medical Research (D.A.S.), the University Research Priority Program Integrative Human Physiology at the University of Zurich (C.M.M.), Matching Funds by the University of Zurich (S.W., C.M.M.), Sirtris, a GlaxoSmithKline

company (C.M.M.), Hartmann-Müller Foundation (C.M.M.), and the Zurich, Heart House—Foundation for Cardiovascular Research, Switzerland. Funding to pay the Open Access publication charges for this article was provided by the Foundation for Cardiovascular Research, Zurich, Switzerland.

**Conflict of interest:** J.A. is a scientific founder of and consultant to MitoBridge, D.A.S. is a founder and consultant to Metrobiotech and consultant to GlaxoSmithKline.

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